Synopsis

Development and Optimization of Novel Topical Drug Delivery System for Management of Diabetic Foot Ulcer

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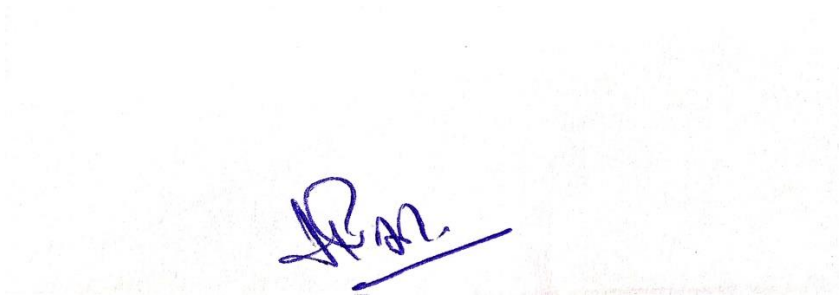

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Introduction

Diabetes mellitus (DM) is metabolic disorders characterized by hyperglycaemia; which may occurs due the imbalance between insulin secretion and insulin resistance. WHO has reported at about 422 million populations is suffering from diabetes and more than 271 million new people will be diabetic by 2045. Around 61.2 million public are diabetic and it is estimated to augment to 101.2 million by 2030 in India (Whiting et al., 2011). At year basis, it is estimated that approximately 2.5% –15% of world-wide health's budget is invested on DM and Diabetic Foot ulcers (DFU) are the major part of this investment. The WHO also reports that diabetic patient has a 25% of life risk to developing DFU (Ebskov B et al., 2004).The main concern is for elder group because this group is more prone to DFU leading to abscess and osteomyelitis (Reed et al., 2004, Noor S et al., 2015). The DFU is one of the chronic diseases that decrease the quality life and becomes major cause of patient hospitalization and at last morbidity (Jupiter et al., 2015).The DFU patients mortality is double than a non DFU diabetic person. The occurrence rate of foot ulcers or infection among diabetic population has reached from 3% to 13% globally (Zhang et al., 2017). Somewhere in the world, it is estimated that at every 30 sec (kumar V.et al.,2019). one lower limb is surgically removed and around 50–70% of amputations are due to diabetic wounds (Everett et al., 2018).

1.1 ETIOLOGY

The aetiology for DFU is multifactorial. The common primary causes are poor glycemic control, calluses formation, improper foot care, mainly peripheral neuropathy, angiopathy, poor blood circulation and dryness in skin etc (Rosyid et al., 2017).

1.2 PATHOPHYSIOLOGY OF DFU

The foot ulcer is defined as a full-thickness wound below the ankle in a diabetic patient, irrespective of duration according to the international consensus on diabetic foot. The DFU is a multifactorial pathophysiological state characterized by infection, deep tissues damaged and ulcer. It is a result of combination of many risk factors including foot deformities, peripheral neuropathy, arterial insufficiency, peripheral vascular disease and trauma

(Yazdanpanah et al., 2015).Usually, the DFU develops in three stages. The initial stage is the development of a callus, results from neuropathy. The motor neuropathy causes physical deformity of the foot, and sensory neuropathy leads to ongoing trauma (Watson et al., 2015). In sensory neuropathy, skin breakdown occurs due to which microbial growth takes place resulting reoccurrence and delay wound healing (Spichler et al.,2015). Dry skin because of autonomic neuropathy is also another causative factor. Hence, Regular trauma of the callus causes subcutaneous haemorrhage and ultimately it turned into an ulcer. Severe atherosclerosis of the small blood vessels in the legs and feet, leads to vascular compromise (Nam et al., 2017). The abnormalities in endothelial function and extended inflammatory response can affect microcirculation, may increase basement membrane thickness or capillary thickness, increased blood coagulation, improper blood flow which is another cause of DFU (Dinker et al., 2013).

1.3 CHALLENGES IN THE TREATMENT OF DFU

A number of dressings, therapies and other treatments are developed by researchers to accelerate healing. The information published by the researcher is not enough to recommend any precise treatment for DFU. There are some current challenges in the management and treatment of DFU (Jeffcoate et al., 2018).

1.3.1 Biofilm formation

It is a multistep process whereby numerous types of microorganisms (Gram positive, Gram negative and fungi) are attached to each other, and hence there is thin film formation take place i.e. called biofilm formation. These microorganisms embedded into a self-produced matrix of extracellular polymeric substance (EPS) (Afonso et al., 2021). EPS contains proteins, glycoprotein, and polysaccharides and confers the ability to adhere the surfaces. Biofilm diversity promotes its survival by creating a thicker biofilm, resulting in more severe infections which are not easy to heal because of resistant to antimicrobials and environmental conditions. The main non healing cause of DFU is the reoccurrence of the infection (Pouget et al., 2020).

1.3.2 Antimicrobial Resistance (AMR)

It is the major cause of non-healing wound. Biofilm-related antimicrobial tolerance involves mechanisms that inhibit the ability of antimicrobials, including both antibiotics and antiseptics, to inflict their action on a susceptible microbe (Olsen I., 2015). On the other side, results from a fundamental change in the microbe itself, leading to the loss of antibiotic effectiveness against a previously susceptible microbe. The excessive exposure of microorganisms to antibiotics promotes to develop AMR (Hurlow et al., 2018). The improper use of effective doses of antimicrobial agents, as may occur during treatment of a DF Infection, may accelerate this process. AMR is currently responsible for higher health care costs associated with treatment and economic losses. So, there is need for a more reliable and cost effective wound management treatment for DFU (Ciofu et al., 2017).

1.3.3 Uncontrolled Infection

Infection, or severe inflammation, is the most common cause of deferred DFU healing despite the numerous antimicrobials is available for management and treatment. The bio-burden in open wounds including DFU presents as a spectrum from contamination through colonization to local and systemic infection (Leaper et al., 2015).

1.3.4 Moisture Balance

It is now commonly accepted that a moist wound environment hastens the healing of wounds and promotes the growth of new tissue. A balanced moist wound environment facilitates cellular growth and collagen proliferation within a healthy non cellular matrix (Junker et al., 2013). On the other hand excess moisture in the wound bed can impair the healing process and damage the surrounding skin (Okan et al., 2007). Inadequate moisture in the wound environment, related primarily to exposure of the wound environment to air, promotes wound desiccation, necrosis, eschar formation and results in poor wound healing rate (Ousey et al., 2016). The development of eschar inhibits the regenerative cells to migrate from the wound fringe into the wound centre. To maintain the moisture level in the wound is very critical (Patel et al., 2019). An appropriate moist environment in the wound promotes cellular growth and the establishment of a provisional wound matrix by stimulating the action of cytokines, growth factors, and chemokine's. Hence, it is not easy to maintain the suitable moisture environment in the wound bed (Tsourdi et al., 2013).

1.4 CURRENT AVAILABLE TREATMENT FOR DFU

DFU management necessitates the accurate categorization of phase and intensity. Adequate treatment for DFUs must involve an emphasis on DM management as well as surgical wound, proper infection prevention and control, pressure relief, and blood circulation optimization (Alexiadou et al., 2012). The fundamental care for treatment and control of DFU is centred on the administration of sufficient perfusion, pressure reduction, and infection prevention as well as debridement. Other types of treatments for DFUs were applied as technology has advanced, like the production of skin analogues, negative pressure wound therapy, hyperbaric oxygen, the creation of novel dressings that incorporate growth hormones, and the usage of tissues created through bioengineering (Howell et al., 2005). Growth factors are a major technological advance that is used for wound healing. The FDA approved growth is PDGF-BB and granulocyte colony-stimulating factor (Barrientos et al., 2008). Silver based dressing such as Acticoat, are also used useful in the treatment of diabetic foot ulcer (Huang et al., 2021).

1.5 GAPS IN THE CURRENT TREATMENTS

In diabetic wounds, the persistence of inflammation stage results in continuous influx of neutrophils, which cause production of excess amount of pro-inflammatory cytokine like (TNF-α) and down regulation of the anti-inflammatory cytokine like inter- leukin-10. This condition of wound also stimulate the free radicals generation, which further exaggerate the continual inflammatory condition in diabetic ulcer (Kant et al.,2021). The currently available treatment focuses only on the antimicrobial effect but they do not focus on the:

a) Inflammation of the wound
b) Reactive oxygen species (ROS) formation
c) Fibroblast and granulocytes formation
d) Re-epithelization of the wound

So, in order to bridges these gaps we aim to evaluate Quercetin in addition with the standard antimicrobial agents i.e. silver. QCT has been found to stimulate angiogenesis and proliferation of epithelial cells and fibroblasts. QCT decreased inflammatory cells, and increased fibroblast proliferation, re-epithelization and quality of healing in diabetic

wounds. It also helps to decrease tumor necrosis factor alpha (TNF-a), Interleukin 1 beta (IL-1b), and increased Interleukin 10 (IL-10), vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-b1) expressions and elevates the blood vessels formation and promotes the neuronal regeneration. QCT stimulate the switching of fibroblast to myofibroblast as well as synthesis of good collagen fibers in diabetic wound (Hatahet et al., 2016).

So, to fill these gaps in this thesis, aim to development of an efficacious, efficient and cost effective product that can have advance wound healing capacity without any dermal toxicity to treat the diabetic foot ulcer. In tend to make a prototype of (QCT & AgNPs) which is cost effective with closed Target Product Profile (TPP) and commercially scalable must have the following requirements.

The DFU product must have a number of requirements:

a) It should not be toxic

b) It should provide a proper moisture to the wound

c) It should be patient compliance i.e. easy to apply and remove

d) It should have an appropriate contact time at the site of application

e) It should be cost effective

REVIEW OF DIABETIC FOOT ULCER

Diabetic foot ulcer (DFU) is one of the most common and severe complications of diabetes mellitus (Armstrong DG et al., 2017). Due to local and systemic factors, the healing process does not progress towards proliferation and maturation phases. Severe infection inhibits the synthesis of cytokines, proteins, and growth factors which affects the proliferation, migration of fibroblast and keratinocytes formation (Edwards et al., 2004). Ulcers are usually in the areas of the foot which encounters repetitive trauma and pressure sensations. *S.aureus*, *E.coli and P. aeruginosa* are the common infective organisms in this disease. DFU is the leading cause of morbidity, amputation and mortality (Jneid J et al., 2017).

Management of DFU includes wound debridement, offloading the ulcer's extracellular matrix protein (ECM) extracts, bioengineered skin substitutes, negative pressure therapy, hyperbaric oxygen and growth factors such as platelet derived growth factor, epidermal growth factor and fibroblast growth factors (Everett et al., 2018). But the overall approaches are focused on closure of wounds rather than skin regeneration that leads towards re-epithelialization. The re-epithelialization process can be separated from infective wound process because

sometimes re-epithelialization is occurred but the dermis and adjacent tissues are not healed still completely, that stimulates the reoccurrence of infection and inflammation resulting again wound formation

(Gosain et al., 2004).

So, research on DFU is the need of the hour due to prevalence of diabetes in world. Multiple approaches are being studied by the researcher but the effective delivery of the drug on the wound site is limited. Demands for the new technologically products for treatment of DFU is rising globally. So, we focus on the development of a topical delivery system incorporated with flavonoid because novel drug delivery systems loaded with flavonoids is the most preferred these days, due to minimum side effects, less toxicity, increased solubility and bioavailability (Nagula et al., 2019). It also helps in providing site specific delivery with better pharmacokinetic parameters. Flavonoids possess wide range of pharmacological properties such as anti-inflammatory, antioxidant, apoptosis and antiproliferative (Saraf et al., 2013). All these activities can be directly linked to the healing of wound. Flavonols act as antioxidants and protects reactive oxygen species (ROS) formation (Wang et al., 2020). Quercetin (QCT) is a flavonols abundantly found in leafy vegetables, citrus fruits, berries etc. QCT reduces inflammation by decreasing the edema, leukocyte formation and irritation. It triggers tissue regeneration by promoting the growth of new collagen fibers and producing ground substance to restore the skin structure (Vedakumari et al., 2017). Also, QCT inhibits various inflammatory mediators such as interleukins (IL), prostaglandins (PGs), produced by COX and LOX (Gallelli et al., 2020). Nitric oxide (NO) is one of the inflammatory mediators synthesized from nitric oxide synthase which generates reactive nitrogen species such as peroxynitrite. QCT helps in inhibiting all the mediators which causes oxidative stress. It prevents cell death by inhibiting caspase-3 pathway and decreases the level of histidine decarboxylase, IL-6, by inhibiting the mast cell secretion (Park et al., 2015). Few research studies of QCT loaded formulation for the DFU treatment are mentioned in Table No. 1.

Table No. 2.1: QCT based developed formulation for the treatment of DFU

Research conducted	Results	Reference
Wound healing potential of chitosan-based QCTNPs was investigated using ionic gelation method	QCT loaded NPs improved wound healing by modulation of cytokines and growth factors	(Choudhary et al., 2020)
Fibrous Polycaprolactone/Graphene	QCT enhanced the overall	(Faraji et al.,

oxide/QCT nanocomposite was fabricated with the help of electro spinning	antibacterial action by reducing bacterial growth by about 50% after 12 h. This dressing has potential for optimal wound healing	2020)
Chitosan–fibrin composite (CF) scaffolds impregnated with QCT (Q) were developed for diabetic wound healing applications	Q-CF scaffolds exhibited good bactericidal activity against E.coli and S. aureus. *In- vivo* wound healing experiments revealed that topical application of Q-CF scaffold can significantly accelerate the process of wound healing	(Vedakumari et al., 2017)
QCT-loaded liposome by the thin film hydration method was formulated. The wound healing potential of developed QCT-liposome was evaluated on diabetic rats.	QCT-loaded liposome depicted sustained release of drug in wound areas. Results showed better wound healing efficacy in experimental animals. Hence this preparation can be effectively employed in wound treatment	(Jangde et al., 2016)
Topical hydrogel system was incorporated with QCT, highly skin-permeable growth factors and oxygen carriers. Further, hydrogel was evaluated for diabetic wound healing efficacy	The designed formulation improved re-epithelialization, thicker and mature granulation tissue formation, and more extensive collagen deposition was noticed	(Jee et al., 2019)

The studies of flavonoids clearly depicted its wound healing potential, but in the treatment of DFU effective treatment strategies should be incorporated for optimal wound healing. Hence, bioflavonoid must be blended or loaded with other therapeutic agents possessing antimicrobial properties. Silver nanoparticles (AgNPs) are considered promising option for

the treatment of DFU due to its strong antimicrobial action (Ouay et al., 2015). The greatest barrier to healing is represented by the biofilm, which is highly resistance to conventional antimicrobial therapies (Kalishwaralal et al., 2010). Due to intrinsic therapeutic properties and multi-site action, AgNPs exhibit a broad-spectrum antibacterial capability against many micro-organisms and demonstrate a huge potential to overcome the emerging issues in the area of microbial resistance in different applications (Salomoni et al., 2017). AgNPs has received significant attention because of the emergence of antibiotic-resistant strains and its low tendency to develop resistance (Beyth et al., 2015). AgNPs has led to increased demand for its medical applications (Burdușel et al., 2018), including wound dressings. Therefore, recent researches are focus on the development of new and effective wound care materials. The nano particles are also well-known to stimulate the healing process by facilitating suitable movement through the different healing phases. To date, focuses have been made on the efficacy of AgNPs in treating the diabetic wound, where these NPs are known to exploit potential biological properties in producing anti-inflammatory and antibacterial activities. AgNPs are also known to activate cellular mechanisms towards the healing of DFU (Senthil et al., 2017). Table No. 2 explains the details of researches conducted using AgNPs for through understanding of role of AgNPs in DFU treatment.

Table No. 2.2: AgNPs loaded formulation for DFU Treatment

Research Conducted	Results	Reference
Developed an antimicrobial peptide-AgNPs composite and have tested its wound healing properties *in -vivo* on a diabetic rat model	The results showed improved wound healing without side effects on dermal tissues and enhanced interactions between peptide and lipopolysaccharide moieties on bacteria, thus also indicating a wide-spectrum activity without inducing bacterial resistance	(Dai et al., 2016)
The wound healing property of AgNPs was tested on wound models in albino rats. The experimental animals were treated with developed cream containing diverse percentages of AgNPs and at the	The result of this study concluded the reduced wound area, increased collagen deposition, few macrophages, tissue edema, and more fibroblasts formation. Hence in this study, potential wound was noticed by	(Parveen et al., 2018)

highest percentage of silver	application of AgNPs.	
Developed biosynthesized AgNPs based c and tested on diabetic rats.	The findings from the study represented the efficacy of AgNPs in wound healing activity and enhanced percent of wound contraction in experimental animals (rats)	(Gong et al., 2018)
Nano biocomposites impregnated with AgNPs were tested on STZ induced diabetic mice model	The results from this experiments showed accelerated wound healing through significantly increased expression of collagen and growth factors, improved re-epithelialization, vasculogenesis and collagen deposition compared to control groups	(Singla et al., 2017)
Formulated a complex of AgNPs and aqueous curcumin: hydroxy propyl-β-cyclodextrin and loading them into bacterial cellulose hydrogel dressing. Further, this delivery system was tested for diabetic wound healing efficacy	These novel AgNPs dressing exhibited antimicrobial activity against three common wound-infecting pathogenic *microbes S. aureus, P. aeruginosa and Candida auris*	(Gupta et al., 2020)

Rationale & Objectives

OBJECTIVES:

- **a.** To enhance the solubility of QCT.
- **b.** To minimize the dermal toxicity of AgNPs.
- **c.** To enhance the efficacy of formulation by combining the QCT and AgNPs.
- **d.** To evaluate the synergistic effect of combination of (QCT and AgNPs).
- **e.** To study the antimicrobial potential of QCT, AgNPs and combination of (QCT and AgNPs) on *S.aureus* and *E.Coli*.

f. To evaluate and compare the skin irritation effect of optimized formulation with marketed Ag gel on mice skin.

g. To evaluate and compare the therapeutic efficacy of optimized QCT-AgNPs emulgel on STZ induced diabetic mice excision wound model.

h. To assess the stability studies of the QCT, AgNPs and optimized QCT-AgNPs emulgel.

THE RATIONAL FOR SELECTION OF QCT

In chronic wounds such as DFU the inflammatory and hypoxia environment stimulates the Reactive Oxygen Species (ROS) production. These species further causes cell damage and become leading cause of proteases and inflammatory cytokines stimulation. Therefore, applying strong antioxidants may reduce ROS to normal levels, preventing wounds from becoming chronic. Quercetin (QCT) is a naturally occurring potential flavonoid and has antiulcer, anti-inflammatory and anti-infective activities due to its ability to scavenge oxygen free-radicals and inhibit lipid peroxidation. Therefore, QCT is a capable topical ulcer healing agent because of its capacity to increase fibroblast proliferation, decrease immune cell infiltration, and adjust activity in fibrosis-associated signalling pathways . QCT is a lipid-soluble agent and its low water solubility, diffusivity and molecular size may account its poor skin penetration ability. So, we developed QCT-SMEDDS. SMEDDS is a potential approach to enhance solubility of drug resulting increased retention time site of action and stability of drug that ultimately help in early healing of wound.

RATIONALE FOR SELECTION OF SILVER NANOPARTICLES (AgNPs)

Nanoparticles exhibit novel properties which sharply depend on their size, surface area and morphology which help them to interact with plants and microbes. The advent of nanotechnology, and combining the bactericidal silver with nano form such as AgNPs, has made its applicability in various fields. The AgNPs have exposed excellent bactericidal properties against a wide range of wound containing microorganisms. The bactericidal mechanism of action of AgNPs has not been understood completely yet, but it is concluded in number of studies that AgNPs are effective against Gram positive and Gram negative bacteria those are found in wound bed. They may damage both the bacteria cell wall and plasma membrane and also can inhibit DNA replication. Moreover, the antimicrobial effect also depends upon the method of preparation, nanoparticle size, shape, surface area and sort of reducing agents and concentration used for nanoparticles synthesis. There is a continuous increase in the number of multi-drug resistant bacterial strains due to mutation, pollution and

changing environmental conditions. To circumvent this predicament scientists are trying to develop drugs for the treatment of such microbial infections. So, AgNPs have been found to be very effective against many infectious bacteria and hence attained an important place as an antimicrobial agent from time immemorial. They are useful in acute and chronic infection.

References

Afonso, Ana C., Diana Oliveira, Maria José Saavedra, Anabela Borges, and Manuel Simões. 2021. "Biofilms in Diabetic Foot Ulcers: Impact, Risk Factors and Control Strategies." International Journal of Molecular Sciences 22 (15). https://doi.org/10.3390/ijms22158278.

Agrawal, Sarita, Tapan Kumur Giri, Dulal Krishna Tripathi, and Ajazuddin Alexander. 2012. "A Review on Novel Therapeutic Strategies for the Enhancement of Solubility of Hydrophobic Drugs through Lipid and Surfactant Based SEDDS : A Novel Approach." American Journal of Drug Discovery and Development.

Aithal, Gururaj C., Usha Yogendra Nayak, Chetan Mehta, Reema Narayan, Pratibha Gopalkrishna, Sudharsan Pandiyan, and Sanjay Garg. 2018. "Localized in Situ Nanoemulgel Drug Delivery System of Quercetin for Periodontitis: Development and Computational Simulations." Molecules 23 (6). https://doi.org/10.3390/molecules23061363.

Aiyalu, Rajasekaran, Arulkumaran Govindarjan, and Arivukkarasu Ramasamy. 2016. "Formulation and Evaluation of Topical Herbal Gel for the Treatment of Arthritis in Animal Model." Brazilian Journal of Pharmaceutical Sciences 52 (3): 493–507. https://doi.org/10.1590/s1984-82502016000300015.

Akula, Samatha, Aravind Kumar Gurram, and Srinivas Reddy Devireddy. 2014. "Self-Microemulsifying Drug Delivery Systems: An Attractive Strategy for Enhanced Therapeutic Profile." International Scholarly Research Notices: 1–11. https://doi.org/10.1155/2014/964051.

Alexiadou, Kleopatra, and John Doupis. 2012. "Management of Diabetic Foot Ulcers." Diabetes Therapy 3 (1): 1–15. https://doi.org/10.1007/s13300-012-0004-9.

Ali Khan, Barkat, Shafi Ullah, M. Khalid Khan, Sultan M. Alshahrani, and Valdir A. Braga. 2020. "Formulation and Evaluation of Ocimum Basilicum-Based Emulgel for Wound Healing Using Animal Model." Saudi Pharmaceutical Journal 28 (12): 1842–50. https://doi.org/10.1016/j.jsps.

Anwer, Md Khalid, Mohammed A. Al-Mansoor, Shahid Jamil, Ramadan Al-Shdefat,

Mohammad Nazam Ansari, and Faiyaz Shakeel. 2016. “Development and Evaluation of PLGA Polymer Based Nanoparticles of Quercetin.” International Journal of Biological Macromolecules 92 (March 2018): 213–19. https://doi.org/10.1016/j.ijbiomac.

Armstrong, David G., Andrew J.M. Boulton, and Sicco A. Bus. 2017. “Diabetic Foot Ulcers and Their Recurrence.” New England Journal of Medicine 376 (24): 2367–75. https://doi.org/10.1056/nejmra1615439.

Babavalian H1, 2, Tebyanian H2, 3, Latifi AM2, Shokrgozar MA4, Bonakdar S4, and Shakeri F2. 2015. “Diabetic Foot Ulcers and Their Recurrence.” Bratislavsk?? Lek??Rske Listy 116 (5): 296–301. https://doi.org/10.4149/BLL.

Barrientos, Stephan, Olivera Stojadinovic, Michael S. Golinko, Harold Brem, and Marjana Tomic-Canic. 2008. “Growth Factors and Cytokines in Wound Healing.” Wound Repair and Regeneration 16 (5): 585–601. https://doi.org/10.1111/j.1524-475X.2008.00410.x.

Burdușel, Alexandra Cristina, Oana Gherasim, Alexandru Mihai Grumezescu, Laurențiu Mogoantă, Anton Ficai, and Ecaterina Andronescu. 2018. “Biomedical Applications of Silver Nanoparticles: An up-to-Date Overview.” Nanomaterials 8 (9): 1–25. https://doi.org/10.3390/nano8090681.

Choi, Du Hyung, Yun Sik Kim, Dae Duk Kim, and Seong Hoon Jeong. 2019. “QbD Based Development and Evaluation of Topical Microemulsion-Based Hydrogel against Superficial Fungal Infections.” Journal of Pharmaceutical Investigation 49 (1): 87–103. https://doi.org/10.1007/s40005-018-0386-4.

Ciofu, Oana, Estrella Rojo-Molinero, María D. Macià, and Antonio Oliver. 2017. “Antibiotic Treatment of Biofilm Infections.” Apmis 125 (4): 304–19. https://doi.org/10.1111/apm.12673.

Dai, Xiaomei, Qianqian Guo, Yu Zhao, Peng Zhang, Tianqi Zhang, Xinge Zhang, and Chaoxing Li. 2016. “Functional Silver Nanoparticle as a Benign Antimicrobial Agent That Eradicates Antibiotic-Resistant Bacteria and Promotes Wound Healing.” ACS Applied Materials and Interfaces 8 (39): 25798–807. https://doi.org/10.1021/acsami.6b09267.

Das, Ratan, Siddarth S. Nath, Dipankar Chakdar, Gautam Gope, and Ramendhu Bhattacharjee. 2010. “Synthesis of Silver Nanoparticles and Their Optical Properties.” Journal of Experimental Nanoscience 5 (4): 357–62. https://doi.org/10.1080/17458080903583915.

Aiyalu, Rajasekaran, Arulkumaran Govindarjan, and Arivukkarasu Ramasamy. 2016.

“Formulation and Evaluation of Topical Herbal Gel for the Treatment of Arthritis in Animal

Model." Brazilian Journal of Pharmaceutical Sciences 52 (3): 493–507. https://doi.org/10.1590/s1984-82502016000300015.

Akula, Samatha, Aravind Kumar Gurram, and Srinivas Reddy Devireddy. 2014. "Self-Microemulsifying Drug Delivery Systems: An Attractive Strategy for Enhanced Therapeutic Profile." International Scholarly Research Notices: 1–11. https://doi.org/10.1155/2014/964051.

Alexiadou, Kleopatra, and John Doupis. 2012. "Management of Diabetic Foot Ulcers." Diabetes Therapy 3 (1): 1–15. https://doi.org/10.1007/s13300-012-0004-9.

Ali Khan, Barkat, Shafi Ullah, M. Khalid Khan, Sultan M. Alshahrani, and Valdir A. Braga. 2020. "Formulation and Evaluation of Ocimum Basilicum-Based Emulgel for Wound Healing Using Animal Model." Saudi Pharmaceutical Journal 28 (12): 1842–50. https://doi.org/10.1016/j.jsps.

Armstrong, David G., Andrew J.M. Boulton, and Sicco A. Bus. 2017. "Diabetic Foot Ulcers and Their Recurrence." New England Journal of Medicine 376 (24): 2367–75. https://doi.org/10.1056/nejmra1615439.

Dai, Xiaomei, Qianqian Guo, Yu Zhao, Peng Zhang, Tianqi Zhang, Xinge Zhang, and Chaoxing Li. 2016. "Functional Silver Nanoparticle as a Benign Antimicrobial Agent That Eradicates Antibiotic-Resistant Bacteria and Promotes Wound Healing." ACS Applied Materials and Interfaces 8 (39): 25798–807. https://doi.org/10.1021/acsami.6b09267.

Das, Ratan, Siddarth S. Nath, Dipankar Chakdar, Gautam Gope, and Ramendhu Bhattacharjee. 2010. "Synthesis of Silver Nanoparticles and Their Optical Properties." Journal of Experimental Nanoscience 5 (4): 357–62. https://doi.org/10.1080/17458080903583915.

Dinker R Pai, Simerjit Singh. 2013. "Diabetic Foot Ulcer – Diagnosis and Management." Clinical Research on Foot & Ankle 01 (03): 1–9. https://doi.org/10.4172/2329-910x.1000120.

Edwards, Ruth; Harding, Keith G. 2004. "Bacteria and Wound Healing." Current Options in Infectious Diseases April-Vo (Issue 2-): p 91-96.

Everett, Estelle, and Nestoras Mathioudakis. 2018. "Update on Management of Diabetic Foot Ulcers." Annals of the New York Academy of Sciences 1411 (1): 153–65. https://doi.org/10.1111/nyas.13569.

Ezhilarasu, Hariharan, Dinesh Vishalli, S. Thameem Dheen, Boon Huat Bay, and Dinesh

Kumar Srinivasan. 2020. “Nanoparticle-Based Therapeutic Approach for Diabetic Wound Healing.” Nanomaterials 10 (6): 1–29. https://doi.org/10.3390/nano10061234.

Fu, Jia, Jingjuan Huang, Man Lin, Tingting Xie, and Tianhui You. 2020. “Quercetin Promotes Diabetic Wound Healing via Switching Macrophages From M1 to M2 Polarization.”

Gong, Cui Ping, Shou Cun Li, and Rong Ye Wang. 2018. “Development of Biosynthesized Silver Nanoparticles Based Formulation for Treating Wounds during Nursing Care in Hospitals.” Journal of Photochemistry and Photobiology B: Biology 183 (17): 137–41. https://doi.org/10.1016/j.jphotobiol.

Gosain, Ankush, and Luisa A. DiPietro. 2004. “Aging and Wound Healing.” World Journal of Surgery 28 (3): 321–26. https://doi.org/10.1007/s00268-003-7397-6.

Haider, Mohammed J, and Mohammed S Mehdi. 2014. “Study of Morphology and Zeta Potential Analyzer for the Silver Nanoparticles.” International Journal of Scientific & Engineering Research 5 (7): 381–87. http://www.ijser.org.

Huq, Md Amdadul. 2020. “Green Synthesis of Silver Nanoparticles Using Pseudoduganella Eburnea MAHUQ-39 and Their Antimicrobial Mechanisms Investigation against Drug Resistant Human Pathogens.” International Journal of Molecular Sciences 21 (4). https://doi.org/10.3390/ijms21041510.

Hurlow, Jennifer J., Gavin J. Humphreys, Frank L. Bowling, and Andrew J. McBain. 2018. “Diabetic Foot Infection: A Critical Complication.” International Wound Journal 15 (5): 814–21. https://doi.org/10.1111/iwj.12932.

ICH, 2003. Q1A (R2) Stability Testing of New Drug Substances and Products, In:International Conference on Harmonization, IFPMA, Geneva.” n.d.

Jangde, Rajendra, and Deependra Singh. 2016. “Preparation and Optimization of Quercetin-Loaded Liposomes for Wound Healing, Using Response Surface Methodology.” Artificial Cells, Nanomedicine and Biotechnology 44 (2): 635–41. https://doi.org/10.3109/21691401.

Jee, Jun Pil, Rudra Pangeni, Saurav Kumar Jha, Youngro Byun, and Jin Woo Park. 2019. “Preparation and in Vivo Evaluation of a Topical Hydrogel System Incorporating Highly Skin-Permeable Growth Factors, Quercetin, and Oxygen Carriers for Enhanced Diabetic Wound-Healing Therapy.” International Journal of Nanomedicine 14: 5449–75. https://doi.org/10.2147/IJN.S213883.

Jeffcoate, William J., Loretta Vileikyte, Edward J. Boyko, David G. Armstrong, and Andrew J.M. Boulton. 2018. “Current Challenges and Opportunities in the Prevention and

Management of Diabetic Foot Ulcers." Diabetes Care 41 (4): 645–52. https://doi.org/10.2337/dc17-1836.

Junker, Johan P.E., Rami A. Kamel, E.J. Caterson, and Elof Eriksson. 2013. "Clinical Impact Upon Wound Healing and Inflammation in Moist, Wet, and Dry Environments." Advances in Wound Care 2 (7): 348–56. https://doi.org/10.1089/wound.

Jyoti, Kumari, Mamta Baunthiyal, and Ajeet Singh. 2016. " Characterization of Silver Nanoparticles Synthesized Using Urtica Dioica Linn. Leaves and Their Synergistic Effects with Antibiotics ." Journal of Radiation Research and Applied Sciences 9 (3): 217–27. https://doi.org/10.1016/j.jrras.

Kamboj, Sunil, and Vikas Rana. 2016. "Quality-by-Design Based Development of a Self-Microemulsifying Drug Delivery System to Reduce the Effect of Food on Nelfinavir Mesylate." International Journal of Pharmaceutics 501 (1–2): 311–25. https://doi.org/10.1016/j.ijpharm.

Khan, Abdul Wadood, Sabna Kotta, Shahid Hussain Ansari, Rakesh Kumar Sharma, Amit Kumar, and Javed Ali. 2013. "Formulation Development, Optimization and Evaluation of Aloe Vera Gel for Wound Healing." Pharmacognosy Magazine 9 (36): S6–10. https://doi.org/10.4103/0973-1296.117849.

Khan, Fariba, Md Saiful Islam, Monzurul Amin Roni, and Reza Ul Jalil. 2012. "Systematic Development of Self-Emulsifying Drug Delivery Systems of Atorvastatin with Improved Bioavailability Potential." Scientia Pharmaceutica 80 (4): 1027–43. https://doi.org/10.3797/scipharm.1201-06.

Leaper, D., O. Assadian, and C. E. Edmiston. 2015. "Approach to Chronic Wound Infections." British Journal of Dermatology 173 (2): 351–58. https://doi.org/10.1111/bjd.13677.

Mekkawy, Aml I., Mohamed A. El-Mokhtar, Nivien A. Nafady, Naeima Yousef, Mostafa Hamad, Sohair M. El-Shanawany, Ehsan H. Ibrahim, and Mahmoud Elsabahy. 2017. "In Vitro and in Vivo Evaluation of Biologically Synthesized Silver Nanoparticles for Topical Applications: Effect of Surface Coating and Loading into Hydrogels." International Journal of Nanomedicine 12: 759–77. https://doi.org/10.2147/IJN.S124294.

Mohamed, Loqman A., Nahid Kamal, Khaled H. Elfakhri, Sarah Ibrahim, Muhammad Ashraf, and Ahmed S. Zidan. 2020. "Application of Synthetic Membranes in Establishing Bio-Predictive IVPT for Testosterone Transdermal Gel." International Journal of Pharmaceutics 586 (March): 119572. https://doi.org/10.1016/j.ijpharm.

Nagula, Ruchika L., and Sarika Wairkar. 2019. “Recent Advances in Topical Delivery of Flavonoids: A Review.” Journal of Controlled Release 296 (December 2018): 190–201. https://doi.org/10.1016/j.jconrel.

Park, Eun Joo, Ji Yun Kim, Mi Sook Jeong, Kui Young Park, Kwan Hee Park, Min Won Lee, Seong Soo Joo, and Seong Jun Seo. 2015. “Effect of Topical Application of Quercetin-3-O-(2″-Gallate)-α-l-Rhamnopyranoside on Atopic Dermatitis in NC/Nga Mice.” Journal of Dermatological Science 77 (3): 166–72. https://doi.org/10.1016/j.jdermsci.

Patel, Satish, Shikha Srivastava, Manju Rawat Singh, and Deependra Singh. 2019. “Mechanistic Insight into Diabetic Wounds: Pathogenesis, Molecular Targets and Treatment Strategies to Pace Wound Healing.” Biomedicine and Pharmacotherapy 112 (January): 108615. https://doi.org/10.1016/j.biopha.

Pouget, Cassandra, Catherine Dunyach-Remy, Alix Pantel, Sophie Schuldiner, Albert Sotto, and Jean Philippe Lavigne. 2020. “Biofilms in Diabetic Foot Ulcers: Significance and Clinical Relevance.” Microorganisms 8 (10): 1–15. https://doi.org/10.3390/microorganisms8101580.

Ramaswamy, Shanmugam, Kuppusamy Gowthamarajan, Lalitha Priyanka Dwarampudi, Mahendran Bhaskaran, and Madhuri Kadiyala. 2021. “Analytical Method Development, Validation and Forced Degradation Studies for Rutin, Quercetin, Curcumin, and Piperine by RP-UFLC Method.” Drug Development and Industrial Pharmacy 47 (4): 562–68. https://doi.org/10.1080/03639045.

Rautela, Akhil, Jyoti Rani, and Mira Debnath (Das). 2019. “Green Synthesis of Silver Nanoparticles from Tectona Grandis Seeds Extract: Characterization and Mechanism of Antimicrobial Action on Different Microorganisms.” Journal of Analytical Science and Technology 10 (1). https://doi.org/10.1186/s40543-018-0163-z.

Shafiq, Sheikh, Faiyaz Shakeel, Sushma Talegaonkar, Farhan J. Ahmad, Roop K. Khar, and Mushir Ali. 2007. “Development and Bioavailability Assessment of Ramipril Nanoemulsion Formulation.” European Journal of Pharmaceutics and Biopharmaceutics 66 (2): 227–43. https://doi.org/10.1016/j.ejpb.

Shamloo, Amir, Zahra Aghababaie, Homa Afjoul, Mehrzad Jami, Mina Razaghzadeh Bidgoli, Manouchehr Vossoughi, Ahmad Ramazani, and Kambiz Kamyabhesari. 2021. “Fabrication and Evaluation of Chitosan/Gelatin/PVA Hydrogel Incorporating Honey for Wound Healing Applications: An in Vitro, in Vivo Study.” International Journal of Pharmaceutics 592. https://doi.org/10.1016/j.ijpharm.

Singla, Rubbel, Sourabh Soni, Vikram Patial, Pankaj Markand Kulurkar, Avnesh Kumari, S.

Mahesh, Yogendra S. Padwad, and Sudesh Kumar Yadav. 2017a. “Cytocompatible Anti-Microbial Dressings of Syzygium Cumini Cellulose Nanocrystals Decorated with Silver Nanoparticles Accelerate Acute and Diabetic Wound Healing.” Scientific Reports 7 (1): 1–13. https://doi.org/10.1038/s41598-017-08897-9.

2017b. “In Vivo Diabetic Wound Healing Potential of Nanobiocomposites Containing Bamboo Cellulose Nanocrystals Impregnated with Silver Nanoparticles.” International Journal of Biological Macromolecules 105: 45–55. https://doi.org/10.1016/j.ijbiomac.

Tran, Hien A., Khanh L. Ly, Kate E. Fox, Phong A. Tran, and Thi Hiep Nguyen. 2019. “Immobilization of Antimicrobial Silver and Antioxidant Flavonoid as a Coating for Wound Dressing Materials.” International Journal of Nanomedicine 14: 9929–39. https://doi.org/10.2147/IJN.S230214.

Worachun, N., P. Opanasopit, T. Rojanarata, and T. Ngawhirunpat. 2012. “Development of Ketoprofen Microemulsion for Transdermal Drug Delivery.” Advanced Materials Research 506: 441–44. https://doi.org/10.4028/www.scientific.net/AMR.506.441.

Yadav, Sunil Kumar, Manoj Kumar Mishra, Anupamaa Tiwari, and Ashutosh Shukla. 2016. “Emulgel: A New Approach for Enhanced Topical Drug Delivery.” International Journal of Current Pharmaceutical Research 9 (1): 15. https://doi.org/10.22159/ijcpr.

Yazdanpanah, Leila. 2015. “Literature Review on the Management of Diabetic Foot Ulcer.” World Journal of Diabetes 6 (1): 37. https://doi.org/10.4239/wjd.v6.i1.37.

Yeo, Eileen, Clement Jia Yew Chieng, Hira Choudhury, Manisha Pandey, and Bapi Gorain. 2021. “Tocotrienols-Rich Naringenin Nanoemulgel for the Management of Diabetic Wound: Fabrication, Characterization and Comparative in Vitro Evaluations.” Current Research in Pharmacology and Drug Discovery. 100019. https://doi.org/10.1016/j.crphar.

Zhang, Pengzi, Jing Lu, Yali Jing, Sunyinyan Tang, Dalong Zhu, and Yan Bi. 2017. “Global Epidemiology of Diabetic Foot Ulceration: A Systematic Review and Meta-Analysis†.” Annals of Medicine 49 (2): 106–16. https://doi.org/10.1080/07853890.

Zhang, Xi Feng, Zhi Guo Liu, Wei Shen, and Sangiliyandi Gurunathan. 2016. “Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches.” International Journal of Molecular Sciences 17 (9). https://doi.org/10.3390/ijms17091534.

Zhao, Xin, Peng Li, Baolin Guo, and Peter X. Ma. 2015. “Antibacterial and Conductive Injectable Hydrogels Based on Quaternized Chitosan-Graft-Polyaniline/Oxidized Dextran for Tissue Engineering.” Acta Biomaterialia 26: 236–48.

https://doi.org/10.1016/j.actbio